Comparative assessment of the binding affinity of SARS-CoV-2 variants with hACE2 genotypes in different populations

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1. INTRODUCTION

The coronavirus disease 2019 (COVID-19) was originated in the city of Wuhan, Hubei province, China in late 2019 and rapidly spread across international borders and evolved into a global pandemic by March 2020 presenting substantial challenges to public health with widespread social and economic implication (Mohan & Vinod, 2020). As of February 1, 2022, COVID-19 had impacted over 370 million individuals resulting in a staggering toll of 5,658,702 recorded deaths (Dieter et al., 2022). COVID-19 is caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Coronaviruses comprise a family of genetically related RNA viruses known to induce respiratory tract infections causing respiratory diseases in both mammals and birds which could have mild to fatal outcomes.

Coronaviruses (CoVs) are the largest group of viruses belonging to the Order *Nidovirales*, family *Coronavirinae* and genus *Coronavirus*. The *Coronavirinae* subfamily has been grouped into four genera of viruses – *Alpha (α), Beta (β), Gamma (γ), and Delta (δ) - coronaviruses*, primarily based upon a combination of serological characteristics, genetic relatedness and habitat distinctions (Fehr & Perlman, 2015). Since its emergence, more new variants have evolved, such as Omicron (ο), Epsilon (ε), Zeta (ζ), Eta (η), Theta (θ), Kappa (κ), Lambda (λ), Mu (μ), etc. due to the adaptive mutations in the viral genome over time (Aleem et al., 2024)

The coronavirus family is not new to infectious diseases. These families of viruses infect both humans and vertebrate animals (Ng & Hiscox, 2020). Among these viruses, four types, namely HKUI, NL63, OC43, and 229E, are recognized for causing respiratory diseases. This virus swiftly spread globally, affecting over 200 countries and territories and infecting around 2 million people. SARS-CoV-2 not only targets the respiratory and gastrointestinal systems but

also invades the central nervous system, causing illnesses in humans and various animals such as bats, cattle, birds, and mice (Salata et al., 2019).

Structure:

SARS-CoV-2 is a single-stranded RNA-enveloped virus sharing structural similarities with its predecessors SARS-CoV 1 and MERS (Hu et al., 2020). It contains an enveloped positive-sense RNA virus and four main structural proteins – including spike (S) and membrane (M) glycoproteins, envelope (E) and nucleocapsid (N) proteins. The surface spike (S) glycoproteins give the virus a crown-like appearance, therefore it was name "coronavirus," derived from the Latin word "corona" meaning "crown."

Mechanism of Infection

The cellular infection mechanism of SARS-CoV-2 involves the interaction of spike protein (S) of the virus and the cell surface receptor Angiotensin Converting Enzyme – 2 (ACE2) of the host. SARS-CoV-2 enters the host cell in two primary steps: (i) membrane fusion and (ii) endocytosis. In the first step, the spike protein of SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) receptor, facilitated by its receptor-binding domain (RBD). Subsequently, the virus releases its RNA into the host cell. This positive sense viral RNA is then directly translated into various viral proteins through RNA-dependent RNA polymerase reaction. Following this, these proteins come together to form a replication complex, generating additional viral RNA. The newly synthesized RNA, in turn, assembles with viral proteins to create a new virus. Finally, this new virus is released from the host cell (Florindo et al., 2020).

The S protein of SARS-CoV-2, with a size ranging from 180 to 200 kDa, is characterized by an extracellular N-terminus, a transmembrane (TM) domain embedded in the viral membrane, and a concise intracellular C-terminal segment. In its natural state, the S protein assumes a

metastable, prefusion conformation. However, upon interaction with a host cell, extensive structural rearrangements occur in the S protein, facilitating the virus's fusion with the host cell membrane. When the S protein binds to the receptor, transmembrane TM protease serine 2 (TMPRSS2), a type 2 TM serine protease located on the host cell membrane, promotes virus entry into the cell by activating the S protein (Huang et al., 2020) (Huang et al., 2020).

Target Organs of SARS-CoV 2

Although ACE2 mRNA is known to be present in virtually all organs, its protein expression is largely unknown (Hamming et al., 2004). The ACE2 receptor is predominantly present in human lung and small intestine epithelia and thus, SARS-CoV-2 is more likely to infect the respiratory and gastrointestinal tracts (Gu & Korteweg, 2007; He et al., 2006). ACE2 expression has also been reported in various other tissues, including enterocytes, renal tubules, gallbladder, cardiomyocytes, male reproductive cells, placental trophoblasts, ductal cells, eye, and vasculature making them secondary targets of SARS-CoV 2 (Hikmet et al., 2020). Studies have also reported an elevated risk of heart attack and stroke as a long term effect of COVID-19 infection (Sidik, 2022). Presence of ACE2 on cribriform plate close to the olfactory bulb is suggested to be linked with COVID-19 associated anosmia (loss of smell and taste) caused by inflammation, affecting olfactory function and potentially facilitating the virus's entry into the central nervous system (Burks et al., 2021; Shamsundara & Jayalakshmi, 2023).

2. REVIEW OF LITERATURE

A comprehensive literature review was conducted using online available scientific databases such as PubMed and Google Scholar.

Detailed Structure of Viral Spike (S) Protein

The SARS-CoV-2 spike (S) is a glycoprotein that forms a trimer anchored to the viral membrane. The full-length S protein of the Wuhan-Hu-1 strain had 1273 amino acid residues (Kumar et al., 2022). The S protein is heavily glycosylated, with each protomer containing 22 N-linked glycosylation sites (Zhao et al., 2020). The S protein consists of two non-covalently associated subunits - the S1 subunit that binds ACE2 and the S2 subunit anchors the S protein to the membrane. S1 contains - N-terminal domain (NTD), receptor-binding domain (RBD), and C-terminal domains (CTD1 and CTD2), while S2 includes - fusion peptide (FP) and other machinery necessary to mediate membrane fusion upon infection of a new cell such as fusion-peptide proximal region (FPPR), heptad repeat 1 (HR1), central helix (CH), connector domain (CD), heptad repeat 2 (HR2), transmembrane segment (TM), and the cytoplasmic tail (CT) (Jackson et al., 2022). The S1 domain of the subunit facilitates the binding of the virus to the peptidase domain of the host surface receptor angiotensin-converting enzyme 2 (hACE-2). Meanwhile, the S2 subunit is facilitate the viral attachment and entry processes (Chakraborti et al., 2023). The polysaccharide molecules coats the spike proteins and help to camouflage them, evading surveillance of the host immune system during entry (Watanabe et al., 2020).

The RBD of S protein, containing a receptor-binding motif (RBM), engages with the ACE2 receptor through hydrogen bonds, salt bridges, and hydrophobic interactions. The C-terminal domains (CTDs) are primarily β-structured segments from S1 and part of S2, stabilizing the post-fusion conformation (Zhang et al., 2021).

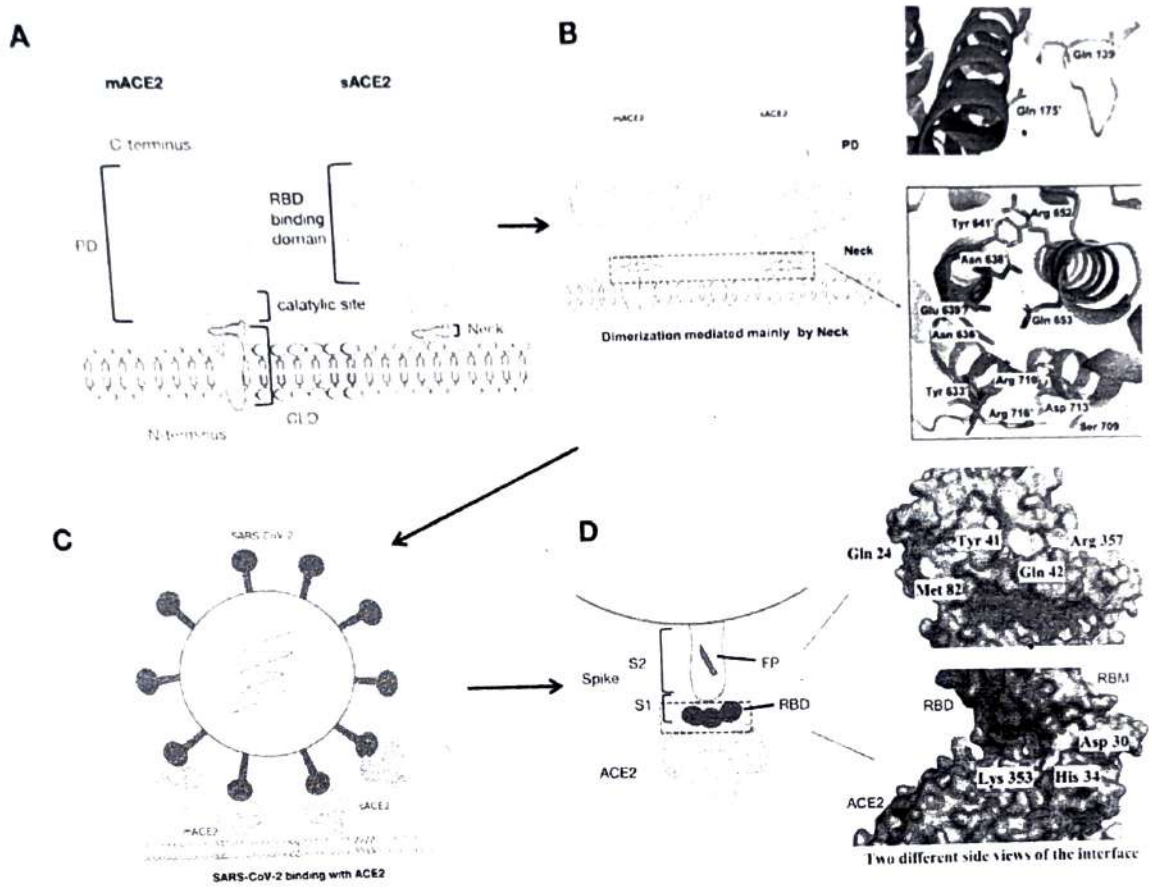

Figure 1: Interaction between SARS-CoV-2 and hACE2 receptor (Feng et al., 2021)

Angiotensin Converting Enzyme 2 (ACE2) and its binding affinity to SARS-CoV 2

ACE2 is a peptidase enzyme found mainly on the cell surface of lungs and kidneys and plays a vital role in regulation of blood pressure through the renin-angioten-aldosterone system (RAAS). It also serves as the primary binding site for SARS-CoV-2. Reports noted hypertension, diabetes, and cardiovascular diseases as prevalent comorbidities in COVID-19 cases, underscoring ACE2's dual role in blood pressure control and viral entry (Bavishi et al., 2020). The ACE2 gene spans 39.98 kb of genomic DNA and contains 18 exons. It maps to chromosome X at position Xp22 and encodes a 100 kDa type I cell-surface glycoprotein with 805 amino acids (Tipnis et al., 2000).

ACE2 structure and its binding with S protein of SARS-CoV-2

Dimer formation is critical, with interactions between the neck domains of two ACE2 protomers and weak forces contributed by Gln139 and Gln175 on the extracellular peptidase domain (PD) dimer. The peptidase domain, within each ACE2 protomer, serves as the site for accommodating the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein. This interaction involves key amino acids, including Tyr41, Gln42, Lys353, and Arg357 at the N terminus; Asp30 and His34 in the middle of the bridge; and Gln24 at the C terminus. Furthermore, the Phe486 of the RBD interacts with the Met82 of ACE2 through van der Waals forces. This intricate molecular architecture underscores the specificity and complexity of ACE2 interactions with the virus (Feng et al., 2021).

ACE2 Polymorphism and COVID 19

Genetic polymorphism refers to differences in DNA sequence among individuals or populations. It could be due to variations in single nucleotide called as single nucleotide polymorphisms (SNPs) or due to repeats, insertions, deletions, and rearrangements of short tandems called as tandem repeat polymorphism. They may arise from mutations, which can be caused by chance or external factors like viruses or radiation, and it is inherited to the next generation (Ismail & Essawi, 2012).

Genetic polymorphisms in the ACE2 gene, such as rs4240157, rs21068809, rs4646155, and rs4830542, have been reported to be associated with hypertension in various populations (Díez-Freire et al., 2006; Pouladi & Abdolahi, 2021; Tikellis et al., 2012).

Drawing parallels with other viruses like HIV (Liu et al., 1996; Samson et al., 1996), MERS-CoV (Kleine-Weber et al., 2020), SARS-CoV 1 (LoPresti et al., 2020), where receptor polymorphisms influenced infection susceptibility, ongoing studies are investigating the

potential impact of ACE2 gene polymorphisms on SARS-CoV-2 on individual and population susceptibility and COVID-19 outcomes. Certain genetic variations in the ACE2 sequence were reported to impact the efficiency of virus cell entry, either by altering its expression levels or increasing the binding affinity for SARS-CoV-2 (Pouladi & Abdolahi, 2021). A study reported a correlation between the allele frequency of rs2285666 and COVID-19 infection rates and case-fatality rates (CFR) in India. The alternate allele (T or A) of rs2285666 gene was found to be negatively correlated with the number of cases and CFR. The study suggests a potential protective role of this genetic variant against COVID-19 however cautioned about the involvement of other genetic elements and external factors in infection rates and CFR (Srivastava et al., 2020).

3. RESEARCH BACKGROUND

Since its emergence, SARS-CoV-2 has profoundly impacted the lives of individuals worldwide, directly or indirectly. The virus has spread extensively, infecting populations across the globe and presenting itself in various new variants. India, in particular, has experienced the presence of several variants, including Alpha (B.1.1.7), Delta (B.1.617.2), Delta plus, Kappa, and most recently, Omicron (B.1.1.529) (Chakraborti et al., 2023). Recently, the World Health Organization has identified JN.1 as a distinct "variant of interest." In India, there have been 1,226 reported cases of this sub-variant, with the highest numbers recorded in Karnataka and Andhra Pradesh (www.ETHealthworld.com, 2024).

In response to this unprecedented situation, scientists worldwide have undertaken an extraordinary effort to understand the virus and its effects. One of the major areas of focus has been the investigation of SARS-CoV-2 infection by entering the host cells, specifically through the interaction between the virus spike (S) protein and its receptor in human, angiotensin-converting enzyme 2 (ACE2) (Watanabe et al., 2020) leading to membrane fusion. The Spike

glycoprotein, which is composed of 1273 amino acids contains N-terminal domain, receptor binding domain (RBD), transmembrane domain, C-terminal domain and cytoplasmic tail apart from few other domains (Loganathan et al., 2020). The trimeric head of the RBD belongs to Spike subunit-1 and directly interacts with the host's ACE2 receptor (Lan et al., 2020).

In a recent report, other than earlier discovered mutations in the RBD domain among Indian isolates, some additional mutations – R408I and E471Q, could significantly modify the overall conformation of the domain resulting in altered binding affinity with the host receptor. Out of 153 SARS-CoV-2 Indian isolates that were studied, 46 contained double mutations with D614G as the common candidate (Chand et al., 2020). It has been hypothesized that D614G mutation helps RBD gain an open conformation leading to better interaction with human ACE2 receptor (Bhattacharyya et al., 2020).

In addition, the rs2285666 polymorphism in ACE2 receptor has been observed to exhibit its effects on populations encompassing European, Iranian, African, etc. (Khalilzadeh et al., 2022). Since the genomic region containing this single nucleotide polymorphism, in particular, has significant associations with an elevated risk and severity of COVID-19 across various populations, therefore, it will be interesting to investigate its potential role in impeding SARS-CoV-2 entry within the individuals of varying ethnicity.

4. OBJECTIVE

On the basis of our previous findings and existing background information, the prime objectives of this study are:

- To retrieve the sequence of ACE2 receptor from different varieties of population.
- To predict the probable structure, of the available ACE2 sequences, in silico.

- To assess the interaction between RBD of various SARS-CoV-2 variants and the corresponding domain of hACE2 belonging to different ethnic regions.
- To investigate the probable reason for difference in pathogenicity among the various population.

5. PLAN OF WORK/ METHODOLOGY

The proposed work shall include *in silico* analysis of the RBD domains of all the SARS-CoV-2 variants and their interaction with ACE2 receptors.

To analyze the spike glycoprotein of various SARS-CoV-2 variants we will examine the spike glycoprotein sequences from NCBI-virus database search and aligning using CLUSTAL OMEGA web servers. Subsequently, we will employ CFSSP (Chou and Fasman Secondary Structure Prediction) to gain insight into potential alterations in spike glycoprotein's structure. By conducting this comprehensive analysis, we anticipate uncovering valuable information pertaining to the structural characteristics of the Receptor Binding Domain within the spike glycoprotein of diverse SARS-CoV-2 variants.

To further enrich our investigation, we will engage in *in-silico* docking experiments, bringing together these domains with ACE2R molecules procured from human genomes of varying ethnic backgrounds, such as European, African, Mongolian, as well as individuals of Indian origin, as per availability in the databases. The results and data derived from these procedures will be compiled and reported, offering a comprehensive understanding of the interaction between SARS-CoV-2 spike glycoproteins and ACE2 receptors across the above mentioned diverse populations.

6. EXPECTED OUTCOME

As the world grappled with the relentless grip of the coronavirus, only a handful of regions emerged victorious in warding off its pernicious effects. This study will throw some light upon the ACE2 receptor genotypes in World population and its interaction with the SARS-CoV-2 spike protein. This endeavor has potential to yield valuable insights into identifying susceptible genotype for COVID-19 and thus, paving the way for proactive preparations in the events of another such potential outbreaks. Furthermore, this study shall pave the path for similar association studies with yet-to-emerge variants of SARS-CoV-2, enabling us to proactively armor ourselves against future uncertainties. This study may also direct us to investigate other possible aspect, such as the immunity among the individuals belonging to a particular ethnic background against viruses, in general. Through this study, we aspire to fortify our defenses and safeguard the well-being of people against any virulent threats ahead.

7. SUMMARY

Variations in the hACE2 receptor and mutations in the RBD domain of SARS-CoV-2 influence their mutual affinity with each other. Our research aims to analyze hACE2 polymorphisms and ACE2-RBD affinity across populations to understand the link between their interaction dynamics and COVID-19 severity, identifying susceptible population and informing proactive measures against such future threats.

KEYWORDS

SARS-CoV-2, Receptor Binding Domain (RBD), angiotensin-converting enzyme 2 (ACE2), *in silico* analysis.

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SIGNATURE OF THE CANDIDATE **SIGNATURE OF THE SUPERVISOR**